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Box 5000
Mayaguez, P.R. 00709

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Hyperimmune mouse IgG made against microbial adhesion enhancement macromolecules (MAEM) reacts specifically with plates coated with immune chicken IgG. Very high titers (100,000) of hyperimmune mouse sera suggest production of antibody(ies) against MAEM antigens, however, the ELISA assay could not discriminate specific from apparent, nonspecific reactions. D-galactose and elevated salt concentrations did not affect this binding. The chicken and mouse may be making antibodies to different portions of the same MAEM or to different MAEM antigens. To clarify this: 1) MAEM antigens from the same source are being isolated independently by toad and chicken immunoaffinity columns. 2) Adhesion enhancement activity of the respective recovered antigens is being assessed. 3) MAEM antigens recovered from the toad immunoaffinity columns are being examined for their quantitative recovery and adhesion activity when they are reacted—

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22a NAME OF RESPONSIBLE INDIVIDUAL: Dr. Michael Marron

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#18. Macromolecular Sea Surfaces

#19 (cont'd) with the immunoaffinity column made with chicken antibodies. (4) Antigens recovered by the two types of columns will be used to produce hyperimmune mouse sera for reexamination of the ELISA specificity. Inhibitory effects of these sera on the adhesion adhancement activity of MAEM antigens will be used to assess similarities among adsorbed MAEM antigens. (AW)

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Code 22
800 N. Quincy Street
Arlington, VA 22217-5000

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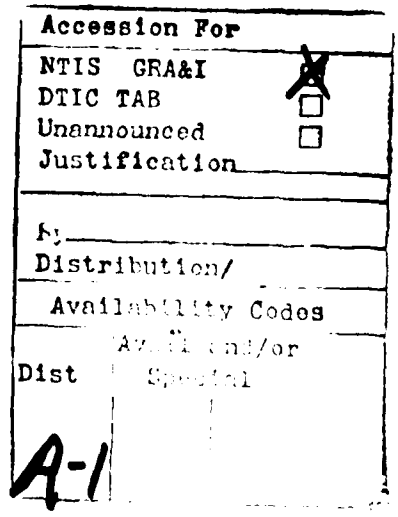

DATE: 1 JUNE, 1989

PROGRESS REPORT ON CONTRACT N00014-88-K-0131

TITLE: The Molecular Specificity of Adsorption of Biofilm Macromolecules and Accumulation of Microbial Biofouling on Artificial Surfaces in the Sea.

PRINCIPAL INVESTIGATORS: T.R. Tosteson and Y. Yamamura. Department of Marine Sciences, University of Puerto Rico-Mayaguez and Microbiology Department, Ponce School of Medicine, Ponce, Puerto Rico.

RESEARCH OBJECTIVES: The diversity and variability of macromolecular components that mediate initial microbial attachment to surfaces in ambient seawater is being determined employing immunological techniques. Antisera against microbial adhesion enhancing macromolecules (MAEM) have been raised in chickens and the immunoglobin-G (IgG) fraction utilized for isolation of crude MAEM from samples of cell free coastal seawater and marine microbial culture media by immunoaffinity chromatography. The objective of the present study is to produce monoclonal antibodies (MAbs) against MAEM and use these to chromatographically isolate and purify individual MAEM from mixtures of such components. Variability and diversity of MAEM produced by biofouling microorganisms, those found soluble in ambient seawater and on biofouled surfaces will be assessed employing a spectrum of MAEM MAbs. The specificity of the interactions of the various MAEM with glass and metallic surfaces will be assessed. Alterations in the physical characteristics of the test surfaces and changes in their susceptibility to microbial biofouling will be correlated with these interactions.

PROGRESS TO DATE: Microbial adhesion enhancing macromolecules (MAEM) have been isolated by immunoaffinity chromatography from samples of cell free coastal seawater and from the laboratory culture growth media of marine microorganisms recovered from biofouled surfaces. MAEM were recovered from these samples by immunoaffinity chromatography employing polyclonal antibodies against these macromolecules. Recovered antigens were pooled for use in the production of monoclonal antibodies (MAbs).

A major objective of this project was to standardize an immune peroxidase ELIZA method suitable for mass screening of hybridomas produced against MAEM antigens. Hens were hyperimmunized by repeated subcutaneous inoculation with PS antigens and their eggs collected. Chicken IgG was isolated using polyethylene glycol and subsequently purified by repeated precipitation with ammonium sulfate. ST plates were individually coated with either normal or immune chicken IgG. Hyperimmune mouse IgG (but not normal mouse IgG) reacted (bound) specifically with immune chicken IgG coated plates, in the presence or absence of MAEM antigen. Thus, the ELIZA method did not appear to be specific. The assay yielded a titer of approximately 100,000 for the hyperimmune mouse serum over that of the normal control. It is important to note that the hyperimmune mouse serum, which was a pool from four mice, consistently showed this property, despite the presence of D-galactose and other simple sugars, as well as elevated salt concentrations.

The success of our approach to this project heavily relies on the specificity of the ELIZA assay. The very high titer of hyperimmune mouse serum suggests that immunization has produced antibody(ies) against MAEM antigens, however, the assay could not discriminate specific from apparent nonspecific

reactions. The specific interaction of immune chicken IgG and hyperimmune mouse sera suggests that either these two animals are making antibodies to different portions of the same MAEM or, more likely to different molecules found in the mixture of MAEM antigens originally recovered from seawater using polyclonal antibodies. MAEM antigens were originally recovered from seawater samples using immunoaffinity columns composed of toad antibodies. These antibodies were raised against the hydroxylapatite (HTP) purified fraction of high molecular weight components found in ambient seawater that specifically enhanced microbial adhesion to surfaces (Tosteson, T.R. et al., Journal of Colloid and Interface Science 104:60-71, 1985). Antigens recovered from the toad antibody column were used to raise antibodies in the chicken in order to construct an additional, larger immunoaffinity column composed of chicken antibodies. Both of these columns, combined in series were used to accumulate MAEM antigens that were initially used in this project. The complex specificity found in the interaction of immune chicken IgG and hyperimmune mouse sera may result from diffrences in the responses of the toad, chicken and mouse to MAEM. Experiments are now in progress to resolve this problem.

The second project objective was to make and isolate hybridomas that produce MAbs against individual PS antigens. To date, 28 hybridoma clones have been shown to produce reactive IgG, detectable using the immune chicken IgG coated plate method described above. These clones are stored frozen in liquid nitrogen, awaiting further refinement of the ELIZA method.

WORKPLAN (remainder of Year 2): The following studies are planned to resolve the ELIZA assay specificity problem: (1) MAEM antigens will be independently recovered from the same source (coastal seawater or microbial culture media) by toad and chicken immunoaffinity columns. The quantity and adhesion activity of the MAEM antigens recovered from the respective columns will be assessed. The quantity and activity of antigens initially recovered from the toad column will be analyzed using the immunoaffinity column made with chicken antibodies. (2) Antigens recovered by both types of columns will be used to produce hyperimmune mouse sera for reexamination of the ELIZA specificity. (3) Inhibitory effects of these sera on MAEM adhesion activity will be examined to compare recovered MAEM antigens. Similar inhibition studies will be conducted with the supernatant solutions recovered from hybridoma clones that produce reactive IgG (i.e. detectable by immune chicken IgG coated plates). (4) HTP purified MAEM will be used directly to produce hyperimmune mouse sera.

INVENTIONS: None.

PUBLICATIONS AND PRESENTATIONS: Molecular Specifity of Microbial Adhesion Enhancing Macromolecules. T.R. Tosteson and Yasuhiro Yamamura. Presented by Y. Yamamura at the Marine Biosurfaces Contractors Meeting, May 20-22, 1989. Hopkins Marine Station, Pacific Grove, California.

TRAINING ACTIVITIES: None.

AWARDS AND FELLOWSHIPS: None.